Appl. No.

: 10/759,785

Filed

January 16, 2004

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph 0013 as follows:

[0001] The invention also provides novel biologically pure cultures of yeasts of the genus *Kluyveromyces*, which have the novel property of being capable of growth in media comprising

cellulose and cellulose derivatives as the sole carbon source. This cellulose or cellulose

derivative may be a variety of soluble or insoluble pure substrates, such as

carboxymethylcellulose, AVICEL® (microcrystalline cellulose), or SIGMACELL® (a high purity

cellulose powder); alternatively, the cellulose or cellulose derivative may be comprised in a

more complex mixture such as the material found in sludges from paper making and recycling,

spent grains from brewing operations, sugared lignin hydrolysates, and corn stover hydrolysates.

In some embodiments, the yeast of the biologically pure culture is of the species Kluyveromyces

marxianus. In a further embodiment, the yeast has the identifying characteristics of K. marxianus

strain SSSJ-0. In another embodiment of the invention, the biologically pure Kluyveromyces

culture is capable of fermenting cellulose or a cellulose derivative to ethanol without the addition

of exogenous cellulases, under either aerobic or anaerobic conditions. In some embodiments, the

culture ferments cellulose or a cellulose derivative to ethanol at about 43 $^{\circ}\mathrm{C}$ in a defined medium

composed of about 20g/L dry weight of cellulose or cellulose derivative, about $0.67\ g/L$ yeast

nitrogen base, and about 0.25 mM magnesium sulfate in a beffer of about 50 mM citrate, pH

about 4.5, and fermentation proceeds until at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%,

80%, 90%, or essentially 100% of the available cellulose is converted to ethanol.

Please amend paragraph 0022 as follows:

[0002] "Cellulose" refers to a linear β 1-4 glucan with the pyranose units in the - 4C_1

conformation, in natural form having a molecular mass between about 50 and 400 kDa.

Processed forms of cellulose may be characterized by a particular degree of crystallization or

polymerization (e.g., AVICEL® or SIGMACELL®)

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Please amend paragraph 0045 as follows:

[0003] Figure 6 illustrates growth (*i.e.*, proliferation) of SSSJ-0 on 2% <u>SIGMACELL®</u> and 2% <u>AVICEL®</u>.

Please amend paragraph 0076 as follows:

[0004] Seed cultures of SSSJ-0 were grown at 43°C in a liquid medium composed of 20g/L glucose, 0.67 g/L yeast nitrogen base, and 0.25 mM magnesium sulfate in a 50 mM citrate buffer, pH 4.5. Mid-log aliquots of the seed culture were inoculated (1:50) into test media of identical composition but containing 20 g/L of the carbon source instead of glucose. The amount of carbon source in the recycled paper sludge was estimated by determining the cellulose content of a dried sample. The cultures were maintained at 43°C in shaking water baths (100 rpm). The increase in cell number was determined by measuring the OD600 of the cultures, or, in the case of the insoluble cellulosics, by plating aliquots of the culture on YPD plates and scoring the number of colonies developed. The doubling time of SSSJ-0 was determined by plotting the number of cells as a function of time in culture.

Carbon Source	Doubling Time (hours)
Glucose	4.0
Arabinose	4.5
Xylose	7.5
Galactose	4.0-4.5
Mannose	4.0-4.5
Cellobiose	3.5
Carboxymethylcellulo	ose 4.0
AVICEL®	10.0
SIGMACELL®	10.0
Recycled paper sludge	e 6.0

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[0005] Seed cultures of SSSJ-0 were grown and inoculated into media containing (1) 2% glucose and 2% L-arabinose; (2) 2% lignin +/- 0.67 g/L yeast nitrogen base (Fisher); (3) 2% corn stover hydrolysate +/- 0.67 g/L yeast nitrogen base; (4) 2% xylose; and (5) 2% SIGMACELL® or 2% AVICEL®, according to the method of Example 3. The increase in cell number was determined by measuring the OD600 of the cultures, or, in the case of the SIGMACELL® (Sigma) and AVICEL® (Fluka), by plating aliquots of the culture on YPD plates and scoring the number of colonies developed. Results of SSSJ-0 growth on 2% glucose and 2% L-arabinose are shown in Figure 2. Results of SSSJ-0 growth on lignin +/- yeast nitrogen base are shown in Figure 3. Results of SSSJ-0 growth on corn stover hydrolysate +/- yeast nitrogen base are shown in Figure 4. Results of SSSJ-0 growth on xylose are shown in Figure 5. Results of SSSJ-0 growth on SIGMACELL® are shown in Figure 6.

Please amend paragraph 0078 as follows:

[0006] Seed cultures of SSSJ-0 were grown and inoculated into media containing plant saccharides according to the method of Example 3, except that the test cultures were grown in air-tight serum bottles to promote anaerobic fermentation. After 48 hours, an aliquot of the medium was withdrawn and enzymatically assayed for ethanol content by incubation with alcohol dehydrogenase and NAD.

Carbon Source	Ethanol (mg/L)
Glucose	67.0
Arabinose	4.6
Xylose	6.4
Galactose	21.2
Mannose	18.9
Cellobiose	3.9
Carboxymethylcellulose	4.6
AVICEL®	2.0
SIGMACELL®	6.1
Brewers spent grain (wet) 5.0
Recycled paper sludge	6.1